Pestic. Sci. 1997, **50**, 127–134

A Study of Fenpropathrin Residues in Tomatoes and Green Beans Grown in Greenhouses in Spain*

M. Martínez Galera, J. L. Martínez Vidal,‡ F. J. Egea González & M. D. Gil García

Department of Analytical Chemistry, University of Almería, 04071 Almería, Spain

(Received 20 August 1996; revised version received 14 November 1996; accepted 5 February 1997)

Abstract: The reduction in residue levels of fenpropathrin with time after treatment of tomatoes and green beans, grown in two types of commercial greenhouse in Almería, Spain, was investigated. Extracted residues were quantified by GC-ECD and their composition verified using GC-MS. Recovery of fenpropathrin from samples spiked with differing amounts of the compound were assessed. For statistical purposes, the loss of fenpropathrin with time was considered to follow a pseudo-first order reaction. The plant species and the season had a significant affect on rates of loss of fenpropathrin but the effect of the type of greenhouse and of the applied dose were not significant.

Key words: fenpropathrin, tomatoes, green beans, diminution, ANOVA, residues, Spain

1 INTRODUCTION

Pesticides play an essential role in the production of our food supply. The American Environmental Protection Agency (EPA), the European Union (EU), CODEX and individual countries[1–5] have published maximum permissible residue levels (MRLs) for pesticides in different commodities. Fruits and vegetables grown in greenhouses need greater amounts of pesticides than those grown out of doors, totalling *c.*50 kg ha^{-1} $year^{-1}$ in greenhouses located on the Mediterranean littoral. In a sample of 84 crops grown in greenhouses in southern Spain (eggplant, squash, green bean, cucumber, pepper, tomato, melon and watermelon), 98 different active ingredients were used against pests, mainly insecticides, acaricides and fungicides.[6]

The use of pesticides on fruits and vegetables is affected by the gradual acceptance of 'Good Agricultural Practices' based on knowledge of the rate of loss of pesticide residues. This process depends, among other things, on climatological conditions, application, species, dose, the interval between application and harvest and the type of greenhouse.

Fenpropathrin is a pyrethroid used as an acaricide insecticide, classified as class II 'moderately hazardous' by the World Health Organisation (WHO),[7] the Admisible Daily Intake (ADI)[8] being 0·03 mg kg^{-1}. Although many papers have been published dealing with the GC determination of pyrethroids,[9,10] there have been few reports of their determination using GC capillary columns.[11] Pyrethroid enantiomers have also been separated using HPLC.[12] The methods of Ambrus and Thier[13] and Specht and Tilkes[14] are those most widely used to extract pyrethroid residues from plants. The actual extraction agent is a mixture of acetone with the water derived from the sample: dichloromethane and hexane are then used for the partitioning step, and the recoveries of several pyrethroids are >70%.

This paper discusses the results obtained in the study of the disappearance of fenpropathrin from tomatoes and green beans grown in greenhouses and the effect that season, kind of greenhouse and applied dose have on the process, in order to determine suitable post-application harvest intervals for these commodities.

* Based on a paper presented at the 1st European Pesticide Residue Workshop 'Pesticides in Food and Drink' held at Alkmaar, the Netherlands on 10–12 June 1996.

‡ To whom correspondence should be addressed.

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

2 EXPERIMENTAL

2.1 Chemicals and solvents

The solvents used were: acetone, hexane and dichloromethane (residue analysis grade, PANREAC, Barcelona, Spain); sodium chloride and sodium sulfate were from the same source. The fenpropathrin standard (99% pure, pestanal quality), obtained from Riedel de Haën (Seelze, Germany), was dissolved in hexane (0·2 mg ml^{-1}) to obtain the primary calibration solution from which solutions of lower concentration were prepared by dilution with n-hexane.

A fenpropathrin 100 g $litre^{-1}$ EC ('Randal', Shell, Spain) was used for treating plants in the greenhouses.

2.2 Apparatus

A Hewlett-Packard (Palo Alto, CA) Model 5890 gas chromatograph equipped with an electron capture detector (ECD-^{63}Ni), a fused silica capillary (HP-1) column containing 100% methylpolisiloxane as stationary phase (12 m × 0·25 mm ID and 0·25 μm film thickness) and an autosampler HP 7673 was used for quantification. HP 3365 Chemstation software was used for instrument control and data manipulation.

A Hewlett Packard Model 5890 Series II gas chromatograph coupled with an HP 5971A mass spectrometer detector and equipped with an on-column injector and an autosampler HP 7673 with HP-UX Chemsystem software was used for GC-MS confirmation purposes. A Chrompak (Middelburg, The Netherlands) CP-Sil 5 capillary column (25 m × 0·25 mm ID and 0·25 μm film thickness) connected to a deactivated fused silica uncoated precolumn (1 m × 0·53 mm ID) was used for the separation.

2.3 GC-ECD operating conditions

These were: injector temperature 250°C; detector temperature 300°C; splitless time 2 min; initial temperature 105°C for 2 min, 20°C min^{-1} up to 150°C, 10°C min^{-1} up to 250°C and then held at 250°C for 5 min. The carrier gas was nitrogen at 0·85 ml min^{-1}, and the same gas at a flow rate of 60 ml min^{-1} was used as make-up.

2.4 GC-MS operating conditions

The initial oven temperature was 60°C for 1 min, then raised at 10°C min^{-1} up to 270°C (5 min hold); on-column injection was used, the initial injector temperature being 63°C and then programmed at the same rate as the oven; helium was used as carrier gas with 55 MPa column head pressure. The mass spectrometer settings were: electron impact ionization mode with 70 eV electron energy, scan mass range m/z 50–400.

2.5 Sampling and storage

For each vegetable, samples were collected at random at 0, 0·5, 1, 2, 3, 4, 5, 8 and 15 days after application of fenpropathrin. Each sample was chopped and divided into four subsamples (50 g) which were stored in individual polyethylene bags at −24°C until extraction.

2.6 Extraction and analysis

The method of extraction used was that published by Luke *et al.*[15] A sample of tomato or green bean (35 g) was shaken mechanically with acetone (100 ml) for 1 h. The mixture was filtered through a filter paper into a 1-litre separating funnel and the filter washed with acetone (2 × 10 ml). Saturated sodium chloride solution (10 ml), hexane (60 ml) and dichloromethane (60 ml) were added and the mixture was shaken vigorously for 2 min, then the organic layers were filtered through anhydrous sodium sulfate (10 g) into a round-bottomed flask. This partition step was repeated twice using hexane (60 ml). The solvent was removed under vacuum at 40°C in a rotary evaporator until almost dry and then just to the point of dryness with a slight nitrogen stream, after which internal standard solution (2 μg ml^{-1}; 0·5 ml) was added and the volume made up to 10 ml with hexane. This solution was injected into the GC-ECD (1 μl) or GC-MS (5 μl).

2.7 Recovery study

The recovery study was carried out by spiking with fenpropathrin standard solutions (100 μl) of fresh tomato and green bean samples (35 g) which had not been treated with the pesticide. The method was assessed at the two different spiking levels, 0·29 and 0·05 mg fenpropathrin kg^{-1} plant material. After evaporation of the hexane using an air-stream, the sample was mixed thoroughly and homogenized for 30 min. The samples were then extracted and analysed. Four replicates of each recovery assay and four blank samples of each vegetable were extracted and analysed.

2.8 Study of diminution of residue level with time

Green beans (cv. Helda) and tomatoes (cv. Daniela) were grown in 0·5-ha plots incorporating 30 000 and 10 000 plants respectively. Fenpropathrin was applied at 1·0 or 0·5 ml formulation $litre^{-1}$ (0·1 or 0·05 g AI

litre^{-1}) and at the rate of 1800 or 2000 litres ha^{-1} in each case. This corresponds to the normal and half doses, respectively. The treatments were carried out in spring (18 May 1994) and winter (17 January 1995). Climatological conditions were monitored and registered during the experiment by using a Jules Richard Model 16352.47 thermohygrographer (Argenteuil-Cedex, France). The experiments were conducted in two different kinds of greenhouses as follows:

1. A flat roof greenhouse of polyethylene (200 μm thickness) with a lateral window 1·30 × 30 m which was covered with a fine netting.
2. An asymmetric-roof greenhouse of polyethylene (200 μm thickness) with a 1·5 × 30 m window in the roof (Fig. 1).

3 RESULTS AND DISCUSSION

3.1 Analysis

Figure 2 shows the ECD chromatogram of fenpropathrin with other pesticides used in the area on these crops and containing dieldrin as internal standard. It can be observed that there are no overlapping peaks in the chromatographic conditions. Figure 3 shows a GC-ECD chromatogram of a hexane extract of green beans spiked with fenpropathrin at 50 μg kg^{-1} and containing the internal standard.

Confirmation of fenpropathrin residues was carried out by GC-MS under conditions described above. Figure 4A shows the mass spectrum at 20·29 min from the total ion current scan chromatogram corresponding to a green bean extract sample taken at $t = 0$ which has a concentration of 1·49 mg kg^{-1} and Fig. 4B is the match of the spectrum for a fenpropathrin standard solution.

3.2 Calibration

Table 1 summarizes the retention time window (RTW) determined for fenpropathrin in the two columns. The RTW is defined as the average of the retention times (eight replicates) plus or minus three times the standard deviation (SD) of retention times (RT).

GC-ECD analysis has been used for quantification. A range of calibration solutions (1 μl) containing 0·05–5·0 μg ml^{-1} of fenpropathrin were injected into the GC-ECD in order to determine dynamic ranges[16] and quantification and detection limits.[17] Table 2 shows the dynamic range of GC-ECD found for the quantification column. The relative standard deviation (RSD) of the response factors (RF) measured between 5 and 100 times the quantification limit (QL) of the pesticide is <9%. The values of detection and quantification limits are 0·010 and 0·035 μg ml^{-1}, respectively.

The calibration data, obtained from eight experimental points by plotting height ratio versus amount ratio,

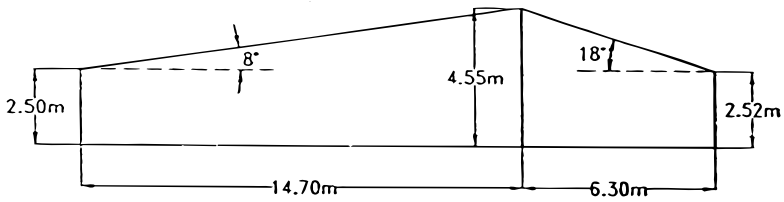

Fig. 1. Structure of an asymmetric-roof greenhouse.

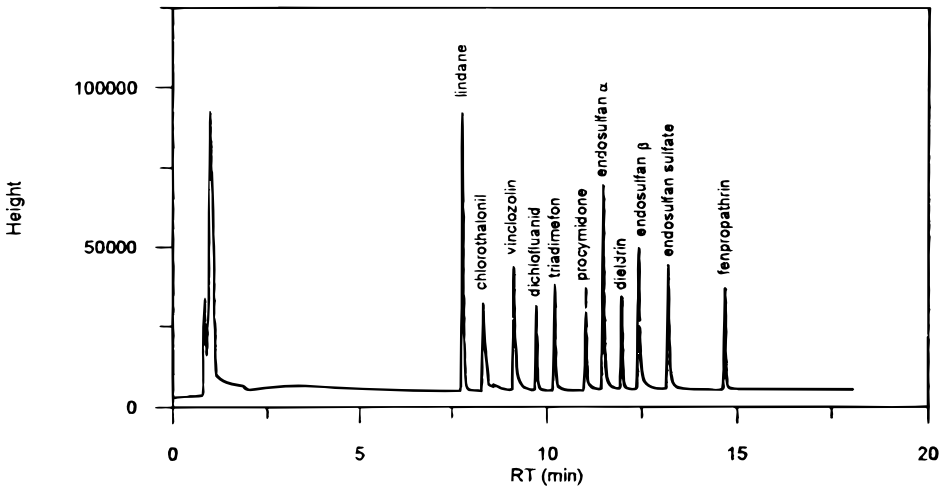

Fig. 2. Chromatogram of a standard mixture of pesticides frequently used in the area.

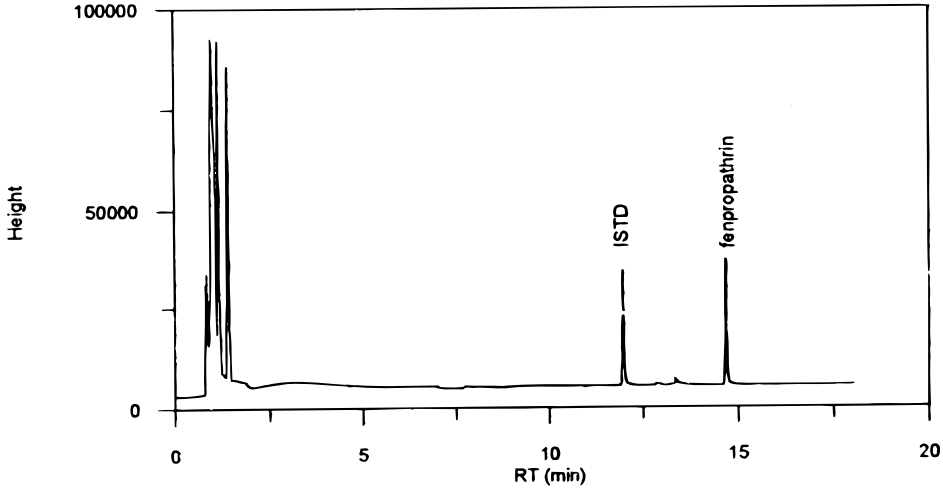

Fig. 3. GC-ECD chromatogram of an extract of green beans spiked with fenpropathrin at 0·050 mg kg^{-1} and containing dieldrin as internal standard (ISTD).

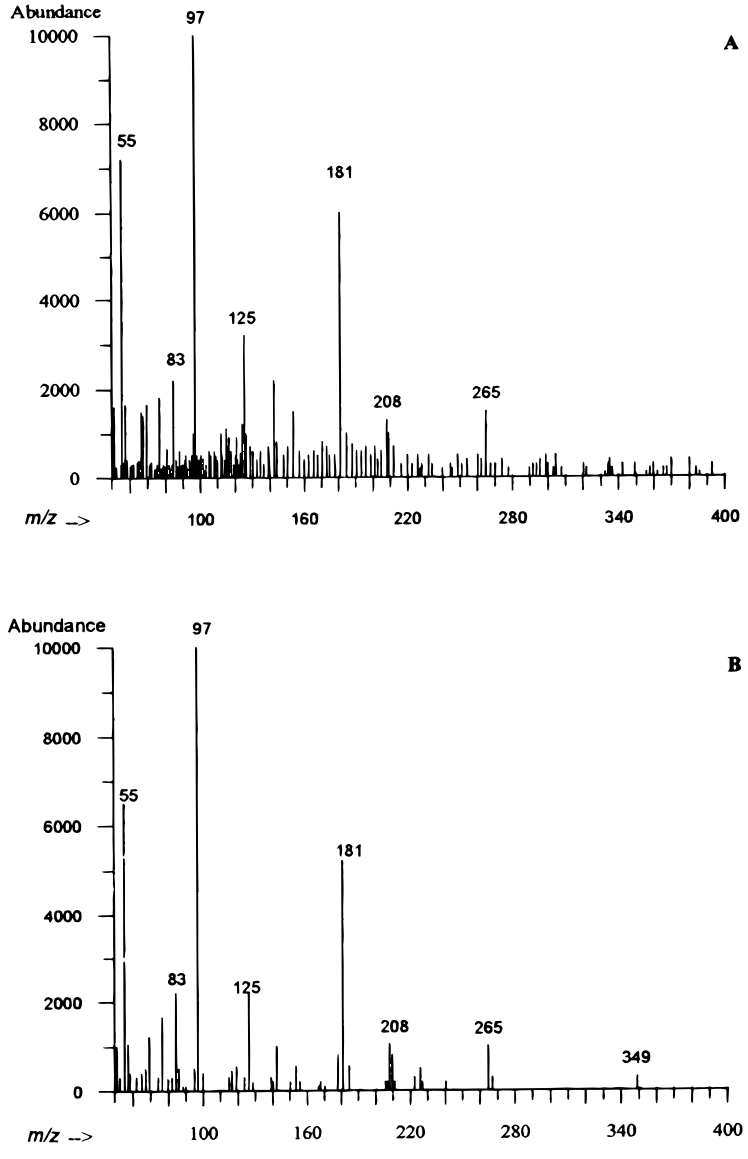

Fig. 4. (A) Mass spectrum from the total ion current scan chromatogram of a green bean sample extract ($t = 0$; $R = 1{\cdot}49$ mg kg^{-1}); (B) match of the spectrum of a fenpropathrin standard solution (5 μg ml^{-1}).

TABLE 1
Retention Times for Fenpropathrin on two Different Columns[a]

	Column HP-1			*Column CP-Sil 5*		
Analyte	*RT*[b]	*SD*[c] × 10^2	*RTW*[d]	*RT*	*SD* × 10^2	*RTW*
Fenpropathrin	14·79	2·0	14·73–14·85	20·29	1·2	20·25–20·33

[a] $n = 4$.
[b] Retention time.
[c] Standard deviation of retention times.
[d] Retention time window.

for fenpropathrin are given in Table 3. Internal standard calibration was used by adding to each calibration point 0·1 μg ml^{-1} of dieldrin.

3.3 Extraction procedure and recovery study

Average recoveries obtained for fenpropathrin in green beans and tomatoes at the two spiked levels are given in Table 4. The values found show that the method is efficient in extracting the residues of fenpropathrin from both vegetables, since the average recoveries are >90% in tomatoes and >87% in green beans with relative standard deviation lower than 5%. The quantification limit[17] of the method was 0·01 mg kg^{-1} for both vegetables.

3.4 Study of diminution of fenpropathrin residue level with time

The effects of season and application rate on the diminution of fenpropathrin residues in green beans grown in flat- and asymmetric-roof greenhouses and in tomatoes grown in a flat-roof greenhouse are shown in Figs 5A, B and C, respectively. The highest level of pesticide was found in green beans grown in winter and in the experiment performed at full dose, while the concentrations found in tomatoes were approximately 50% of those found in green beans.

Statistical interpretation of the loss of fenpropathrin in the experimental plots was performed by assuming that the diminution rate of the residues can be described as a pseudo-first-order reaction according to the equa-

TABLE 2
Dynamic Range for Fenpropathrin Analysed by GC-ECD using an HP-1 Capillary Column

RF[a] *100 × QL*[c]	*RF 80 × QL*	*RF 40 × QL*	*RF 20 × QL*	*RF 1 1 × QL*	*RF 5 × QL*	*RSD* (%)[b]
86	95	99	96	109	105	8·1

[a] Response factor $\mathrm{RF} = \dfrac{\textit{peak height of compound}}{\textit{amount of compound injected}}$.

[b] Relative standard deviation $\mathrm{RSD} = \dfrac{\textit{standard deviation}}{\textit{average of RFs}}$.

[c] Quantitation limit of fenpropathrin.

TABLE 3
Calibration Data for Fenpropathrin Obtained Using 0·1 μg ml^{-1} of Dieldrin as Internal Standard[a]

		Standard deviation	
Equation	*Correlation coefficient*	*Slope*	*Intercept*
F[b] = 0·121(amt ratio) + 0·013	0·9993	0·022	0·026

[a] $n = 4$.
[b] F = signal of fenpropathrin.

TABLE 4
Average Recoveries Obtained for Fenpropathrin in Green Beans and Tomatoes at Two Spiked Levels[a]

Tomatoes			*Green beans*		
Spiked ($mg\ kg^{-1}$)	*Found* ($mg\ kg^{-1}$)	*Recovery* (%)	*Spiked* ($mg\ kg^{-1}$)	*Found* ($mg\ kg^{-1}$)	*Recovery* (%)
0·29	0·26	88·4–94·2	0·29	0·25	83·9–90·9
0·050	0·046	88·9–95·7	0·050	0·045	87·8–93·6

[a] $n = 4$.

tion $R = R_0\ e^{-Kt}$ and can be quantified by a linear semilogarithmic regression analysis $\ln R = \ln R_0 - Kt$, where R is the residue level at t days after pesticide application, R_0 is the residue level at time $t = 0$ and K is the loss rate constant. The regression coefficient (r^2) are >0·98 in all cases.

Statistical and fenpropathrin loss parameters are shown in Table 5. The half-life ($T/2$) range for tomatoes is 3·4–4·2 days and for green beans 4·0–4·5 days. The tenth-life ($T/10$) is between 11·4 and 14·1 days for toma-

TABLE 5
Data for Loss of Fenpropathrin from Treated Green Beans and Tomatoes

Grown	*Season*	*Greenhouse type*	*Dose*	K[a] ($days^{-1}$)	R_0[b] ($mg\ kg^{-1}$)	r^2[c]	$T/2$[d] (*days*)	$T/10$[e] (*days*)	R_{10}[f] ($mg\ kg^{-1}$)
Green beans	Winter	Flat-roof	Normal	0·2	1·29	0·983	4·0	13·4	0·23
			Half	0·2	0·64	0·986	4·2	14·0	0·12
	Spring	Flat-roof	Normal	0·2	0·51	0·998	4·2	13·9	0·10
			Half	0·2	0·26	0·994	4·4	14·5	0·05
		Asymmetric-roof	Normal	0·2	0·70	0·997	4·2	14·0	0·14
			Half	0·2	0·34	0·987	4·5	14·9	0·07
Tomatoes	Winter	Flat-roof	Normal	0·2	0·83	0·986	3·7	12·2	0·13
			Half	0·2	0·33	0·972	3·4	11·4	0·04
	Spring	Flat-root	Normal	0·2	0·27	0·992	3·9	12·9	0·05
			Half	0·2	0·19	0·990	4·2	14·1	0·04

[a] Rate of diminution constant (slope of the linear semilogarithmic regression analysis).
[b] Concentration of fenpropathrin at time $t = 0$ ($\ln R_0$ is the intercept of the linear regression analysis).
[c] Regression coefficient of the linear semilogarithmic regression analysis.
[d] Half-life time of the fenpropathrin residues.
[e] Tenth-life time of the fenpropathrin residues.
[f] Concentration of fenpropathrin at time $t = 10$.

TABLE 6
Analysis of ANOVA for the Loss of Fenpropathrin

	Without interactions		*With interactions*	
Source of variation	F-*value*	*D.F.*[a]	F-*value*	*D.F.*
Species	12·33[b]	1·25	18·65[b]	1·21
Greenhouses	0·06	1·25	2·29	1·21
Season	10·28[b]	1·25	15·54[b]	1·21
Doses	1·93	1·25	0·65	1·21
Species-season			12·15[b]	1·21

[a] Degrees of freedom.
[b] Significant at $P < 0·05$.

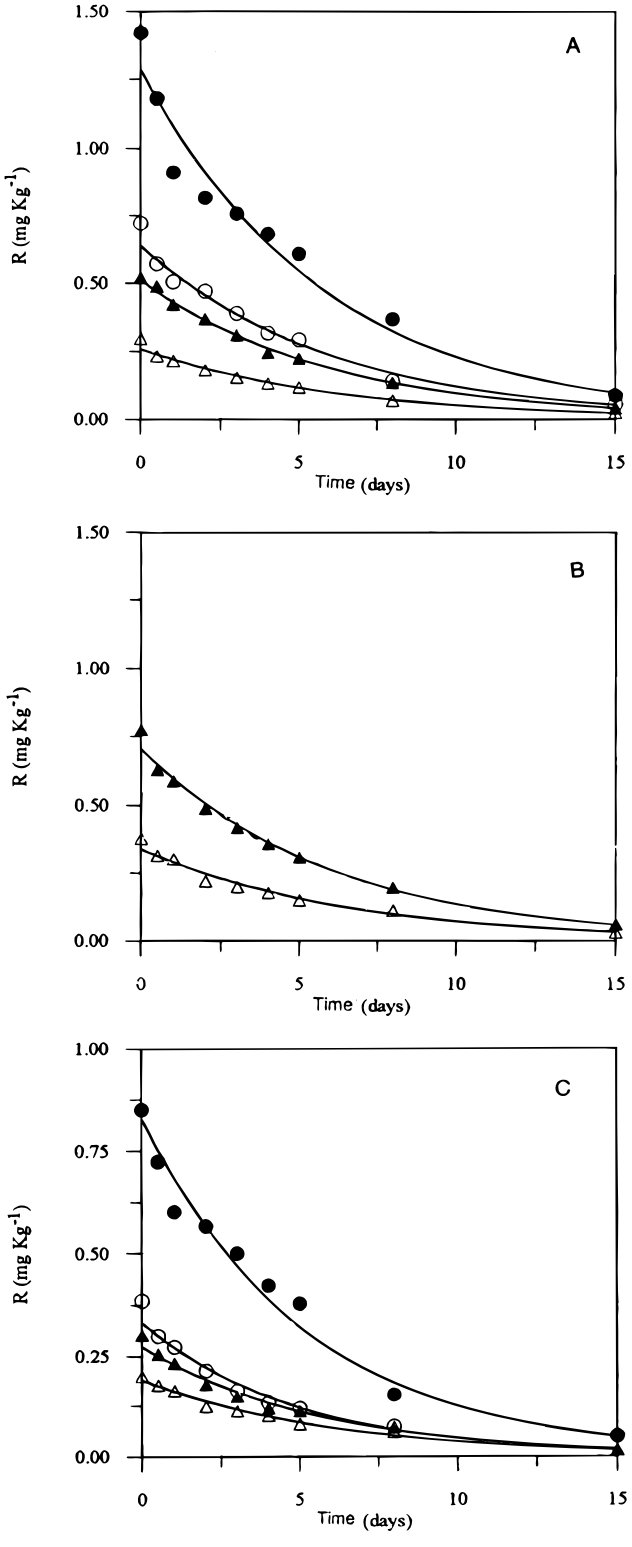

Fig. 5. Diminution of fenpropathrin residues in: (A) green beans grown in a flat-roof greenhouse and (B) in an asymmetric-roof greenhouse and (C) tomatoes grown in a flat-roof greenhouse. (●) Winter, 0·1 g litre^{-1} (○) Winter 0·05 g litre^{-1}. (▲) spring 0·1 g litre^{-1} (△) spring 0·05 g litre^{-1}.

TABLE 7
Average Values of Half-Life Times and Standard Errors for the Treatments

Level	*Average (days) ± Standard error*
Total	4·08 (±0·07)
Tomatoes	3·82 (±0·12)
Green beans	4·25 (±0·05)
Flat-roof	4·00 (±0·07)
Asymmetric-roof	4·38 (±0·08)
Winter	3·84 (±0·11)
Spring	4·24 (±0·05)
Half dose	4·14 (±0·11)
Normal dose	4·06 (±0·07)
Tomatoes—winter	3·51 (±0·09)
Tomatoes—spring	4·13 (±0·12)
Green beans—winter	4·17 (±0·08)
Green beans—spring	4·29 (±0·06)

toes and between 13·4 and 14·9 days for green beans.

Analysis of variance was carried out in order to study the influence on the response 'diminution of fenpropathrin' of the factors: 'species grown' (tomatoes and green beans); 'season' (spring and winter); 'doses' (half and full) and 'type of greenhouse' (flat- and asymmetric-roof), by using the $T/2$ values obtained in each case (Table 6).

The multiple ANOVA indicates that the factors 'species grown' and 'season' have an influence on the loss of fenpropathrin ($F_{crit} = 4{\cdot}24$), whereas the factors 'type of greenhouse' and 'doses' have no significant influence. The cross-effect 'species grown' by 'season' are significant ($F_{crit} = 4{\cdot}33$).

The averages of $T/2$ values and standard errors, either for the total sample or the different factors in the diminution rate of fenpropathrin, are summarized in Table 7. It can be seen that the rate of loss is significantly lower in green beans than in tomatoes and is lower in spring than in winter while for tomatoes in winter the residue level diminution is significantly greater than for the rest of the factors.

The residuals against the different levels of the factors were plotted and there is no reason to suspect lack of homogeneity in the factors, except, perhaps in the factor 'kind of greenhouse', but applying the Bartlet's test[18] ($B = 1{\cdot}073$), there is no significant evidence to reject the homogeneity between the groups.

4 CONCLUSION

According to these values and the maximum residue level given in the Spanish legislature for fenpropathrin in tomatoes (0·50 mg kg^{-1}) and green beans (0·02 mg kg^{-1}), we may conclude that a pre-harvest time can be established for the different conditions

studied. The preharvest time in winter and after applying a normal dose in a flat-roof greenhouse, is three days for tomatoes and more than 15 days for green beans.

REFERENCES

1. *Code of Federal Regulations, Title 40*. US Government Printing Office, Washington, DC, 1988, Part 180.
2. *Official Bulletin of European Communities. Order 93/58/EEC of Conseil*. Bureau of Official Publications of European Communities, Luxembourg, 1993.
3. Anon., *CODEX Alimentarius*, Commission mixte FAO/OMS. Rome, FAO, 1993.
4. Anon., *Pesticides residues in food of plant origin*. Report No 12/1993, National Food Administration, UPPSALA, Sweden, 1992.
5. Order of 27th February 1996 (*Official Spanish Bulletin No. 56, 5 March, 1996*) and *Royal Order 280/1994*, 18th February. Official Spanish Bulletin Bureau, Madrid, Spain.
6. Cabello, T. & Cañero, R., Mezcla de productos empleados en cultivos hortícolas en invernaderos del SE de España. Análisis de costos. *Bol. San. Veg. Plagas.*, **20** (1994) 429–36.
7. Anon., *The WHO Recommended classification of pesticides by hazard*. WHO/PCS.94.2, Geneva, Switzerland, 1994.
8. Anon., *IPCS Summary of toxicological evaluations performed by the joint FAO/WHO meeting in pesticide residues*. WHO/PCS.94.1, Geneva, Switzerland, 1993.
9. Miyamoto, J., The Chemistry, metabolism and residue analysis of synthetic pyrethroids. *Pure Appl. Chem.*, **53** (1981) 1967–2022.
10. Papadopoulou-Mourkidou, E., Analysis of established pyrethroid insecticides. *Residue Rev.*, **89** (1983) 179–208.
11. Bottomley, P. & Baker, P., Multi-residue determination of organochlorine, organophosphorus and synthetic pyrethroid pesticides in grain by gas-liquid and high-performance liquid chromatography. *Analyst.*, **109** (1984) 85–90.
12. Cayley, G. & Sampson, B., Separation of pyrethroid enantiomers by chiral high-performance liquid chromatography. *J. Chromatogr.*, **356** (1986) 123–34.
13. Ambrus, A. & Thier, H. P., Application of multiresidue procedures in pesticide residues analysis. *Pure Appl. Chem.*, **58** (1986) 1035–42.
14. Specht, W. & Tilkes, M., Gaschromatographische Bestimmung von Rückständen an Pflanzenbehandlungsmitteln nach Clean-up über Gel-Chromatographie und Mini-Kieselgel-Säulen-Chromatographie. *Fresenius J. Anal. Chem.*, **301** (1980) 300–7.
15. Luke, M. A., Froberg, J. E., Dosse, G. M. & Masumoto, H. T., Improved multiresidue gas chromatographic determination of organophosphorus, organonitrogen and organohalogen pesticides in produce, using flame photometric and electrolytic conductivity detectors. *J. Assoc. Off. Anal. Chem.*, **64** (1981) 1187–95.
16. Hsu, J. P., Wheeler Jr, H. G., Camann, D. E., Schattenberg, H. J., Lewis, R. G. & Bond, A. E., Analytical methods for detection of nonoccupational exposure to pesticides. *J. Chromatogr. Sci.*, **26**(4) (1988) 181–9.
17. Thier, H. P. & Zeumer, H. (eds), *Manual of Pesticide Residue Analysis*, **1**, Pesticides Commission, DFG, VCH Publishers, Weinheim, Germany, 1987, pp. 37–44.
18. *Statgraphics Reference Manual, Version 5*, Statistical Graphics Corporation, STSC Inc., Rockville, USA, 1995, O24–O25.